Evaluation of recombinant enzyme calibration to harmonized lipoprotein-associated phospholipase A2 activity results between instruments

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Objectives: Enzymatic activity of lipoprotein-associated phospholipase A2 (Lp-PLA2) mediates vascular inflammation in coronary heart disease (CHD). Calibration of Lp-PLA2 activity measurements using a recombinant enzyme was performed to assess intra- and inter-laboratory assay precision and accuracy in routine clinical settings.

Design and methods: Test performance assessment included recovery, analytical sensitivity, linear range, within-lab and site-to-site precision, interference, and analyte stability. Results using the Beckman-Coulter AU400 analyzer were compared to other chemistry analyzers.

Results: Lp-PLA2 activity ranged from 84 to 303 nmol/min/mL in 300 subjects, with 82.0% and 18.0% measurements below and at or above a cut-point of 225 nmol/min/mL, respectively. Results of matched K2-EDTA plasma and serum (n = 131) were similar with a slope of 1.00, y-intercept of 0.05, and R-value of 0.988. Mean recovery ranged from 90 to 106% of baseline after storage at different temperatures and time periods. Limit of detection was ≤10 nmol/min/mL, without deviation from linearity between 10 and 382 nmol/min/mL. Endogenous substances and medications did not interfere with the activity measurements. Overall intra- and inter-laboratory precision among three sites showed coefficients of variation of 3.8% and ±5% respectively. Limit of quantitation was 1.3 nmol/min/mL. Method comparison studies for multiple analyzers demonstrated slopes, intercepts or R2 coefficients ranging from 0.96 to 1.06, −5.6 to 2.0, or 0.997 to 0.999, respectively.

Conclusion: Analytical performance of the calibrated PLAC® test for Lp-PLA2 enzyme activity assay in CHD resistant to a wide variety of pre-analytical factors, with site-to-site reproducibility on multiple analyzers sufficient to standardize results in diverse laboratory settings.

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1. Introduction

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a circulating enzyme whose activity contributes to atherosclerotic cardiovascular diseases (CVD) [1,2] and may be assayed by its cleavage of synthetic substrates containing the sn-2 position of endogenous phospholipid substrates [3]. Lp-PLA2 contributes to plaque development by cleaving oxidized LDL to yield lysophosphatidylcholine and oxidized free fatty acids, which are pro-inflammatory and pro-apoptotic to the atheromatous environment of the arterial wall [3–5]. Clinical studies have demonstrated that Lp-PLA2 enzyme activity is useful in patient management since levels predict CVD events associated with coronary heart disease (CHD) and ischemic stroke, not only in patients with risk factors alone (primary prevention), but also in those with established disease (secondary prevention) [6]. The Lp-PLA2 Studies Collaboration analyzed 32 studies and highlighted differences in Lp-PLA2 enzyme activity levels, which varied primarily due to the use of different substrates [6]. These data suggest that clinical use of Lp-PLA2 enzymatic activity measurements for patient management may be optimized by assay harmonization.

Inter-laboratory agreement of enzyme activity measurements is a goal in laboratory medicine due to variability in results among different laboratories [7]. Enzymes are a special class of analyte requiring standardization of measurements since catalytic activity depends on testing conditions, and measurements may vary substantially between laboratories due to changes in reaction conditions. Such variability may
diminish result comparability and hinder clinician interpretation of results [7], particularly in the context of published assay performance data. The magnitude of activity measurements depends on the components of the reaction system and the analytical conditions, but may be optimized by use of a reference measurement procedure. Two tests for Lp-PLA2 measurements include the same recombinant Lp-PLA2 (rLp-PLA2) protein [8–11] for calibrating measurements of enzyme concentration by ELISA [12] or enzyme activity by colorimetric detection of cleaved synthetic substrate. An additional requirement for enzyme activity standardization is a reference interval based on use of a reference measurement system and clinical populations with similar characteristics to obviate potential influences due to ethnicity and/or environment [7].

The clinical value of Lp-PLA2 activity measurements depends on broad integration of knowledge of the enzyme’s physiology, inflammatory role, demographic variation, and clinical practice, together with interpretation of assay results optimized for CVD outcomes. In this work, we report the use of a recombinant Lp-PLA2 enzyme to calibrate results of the PLAC® Test for Lp-PLA2 activity, which utilizes an activity cut-point of 225 nmol/min/mL targeted for use in primary prevention of CHD. The objective of this study is to demonstrate the analytical performance of the activity assay, including assay precision, sensitivity, linearity, specimen stability, and site-to-site variability, thus satisfying requirements [7] for use as a standardized enzyme activity assay on a variety of automated chemistry analyzers in diverse clinical laboratory settings.

2. Materials and methods

2.1. Specimens

Human plasma and serum were obtained as archival or fresh samples from vendors (Bioreclamation IVT, LAB Express, and ProMedDx) who collected blood samples from consented donors, or as prospective samples from human volunteers (Diadexus) who provided informed consent, under protocols approved by an Institutional Review Board of participating facilities using standard operating procedures. Demographic information was obtained for each subject and was limited to age, gender, and race; no other clinical information was obtained. Details of specimen handling and storage are provided in the Supplemental information.

2.2. Lp-PLA2 enzyme activity

Measurement of plasma or serum Lp-PLA2 activity was performed with the PLAC Test for Lp-PLA2 Activity (Diadexus) using recombinant Lp-PLA2 (rLp-PLA2) enzyme as a standard for calibration as previously described [9]. rLp-PLA2 was manufactured with proprietary procedures and used to develop a set of master calibrators to standardize each production lot due to the absence of a recognized national reference standard. Patient samples, calibration standards, or high and low controls were added to a reaction buffer containing the substrate, 1-myristoyl-2-(4-nitrophenylsucinyl) phosphatidylcholine. Substrate hydrolysis by Lp-PLA2 yielded 4-nitrophenol, which was measured spectrophotometrically using the Beckman Coulter AU400 clinical chemistry analyzer with optimized, instrument-specific settings at 410 nm and an assay time of 8.5 min. Five rLp-PLA2 calibrators were used to generate a standard curve fit of change in absorbance versus Lp-PLA2 activity level in nmol/min/mL. This standard curve was utilized to determine sample Lp-PLA2 activity.

Calibrated sample results were compared to uncalibrated results using two different lots of substrate. A slope from a non-enzymatic curve of absorbances of known solutions of 4-para-nitrophenol (PNP) plotted by nmol was used to convert uncalibrated results to Activity units. Sample results were calculated by the following equation: Lp-PLA2 activity nmol/min/mL = sample kinetic slope/ PNP slope/0.025 mL.

2.3. Sensitivity and linearity

The limit of blank (LoB) and limit of detection (LoD) were determined using calibrators and native K2EDTA plasma, respectively, using analytical approaches described in CLSI EP17-A2 [13], and limit of quantitation (LoQ) was determined using human plasma and non-human plasma dilutions (see Supplemental information).

To determine assay linearity, Lp-PLA2 activity was measured in triplicate with reagents from three kit lots using a specimen dilution series at 13 different levels for each pair of high and low samples. Three high (2000 nmol/min/mL) and three low (≤10 nmol/min/mL) Lp-PLA2 activity specimens were used from individual donors. To generate high activity specimens for this study, rLp-PLA2 protein was spiked into specimens with high native Lp-PLA2 activity. To generate specimens with low activity, patient specimens with low native Lp-PLA2 activity were diluted with chicken plasma (Bioreclamation IVT, P/N CHPLPLEDTA2) to yield Lp-PLA2 activity values of <10 nmol/min/mL.

2.4. Sample matrix

Freshly drawn blood samples were collected from 21 donors in five different tube types with serum and varying anticoagulant (K2, K3 EDTA and heparin). After collection processing, sample aliquots were stored at 2–8 °C for one day, and then tested in triplicate. For data analysis, the three replicates for each tube type were averaged and then compared to K2–EDTA plasma results obtained without a separator using a calculated percent recovery and Deming regression. The activity range of the samples, 94 to 323 nmol/min/mL, encompassed 95% of the observed expected values range.

2.5. Assay robustness

To determine assay robustness, Lp-PLA2 activity measurements were performed on serum samples from 148 subjects using one reagent lot in the absence or presence of five rLp-PLA2 calibrators ranging from 0 to 400 ng/mL, using two different lots of the substrate, 1-myristoyl-2-(4-nitrophenylsucinyl) phosphatidylcholine.

2.6. Lp-PLA2 enzyme activity cut-point

In order to determine clinical performance for the prognostication of future CHD events, an Lp-PLA2 activity cut-point of 225 nmol/min/mL was established to enable assignment of clinically relevant high or low values. For reference, a value of 225 nmol/min/mL was derived from the JUPITER Study [14] based on determinations of Lp-PLA2 activity using archival plasma samples previously stored at ≤70 °C for this study.

2.7. Specimen stability

To determine the effects of temperature and time on the stability of the Lp-PLA2 activity result, prospectively-collected specimens from 26 subjects were assayed on the day of collection with results used as the “baseline”; then these same samples were analyzed after they were stored at different temperatures for varying time periods. To assess the stability of Lp-PLA2 activity in unfrozen or frozen specimens, Lp-PLA2 activity was measured after specimen storage at 4 °C or 26 °C for up to three months or at −20 °C or −70 °C for up to 26 months, respectively. For freeze/thaw study samples, the measured activity value after each freeze thaw cycle was compared to the fresh unfrozen sample value. Measurements of Lp-PLA2 activity for all temperatures and time intervals were compared to baseline results to determine average recovery values.
2.8. Interference testing

To determine whether substances may alter the Lp-PLA₂ activity result, measurements were performed to assess both endogenous (albumin, bilirubin (conjugated and unconjugated), cholesterol, hemoglobin, and triglycerides) and exogenous (acetaminophen, acetylsalicylic acid, atorvastatin, clopidogrel bisulfate, diphenhydramine, fenofibrate, lisinopril, metformin, niacin, tolbutamide, warfarin, and vitamin C) substances by spiking each substance into four K₂-EDTA plasma and four serum specimens (see Supplemental Information).

2.9. Reagent stability

Assessments of reagent stability, open bottle stability, and on board analyzer stability were performed to determine their effects on Lp-PLA₂ activity measurements (see Supplemental Information).

2.10. Assay precision

To determine intra-laboratory precision, Lp-PLA₂ activity was measured using four plasma specimens and two kit control samples containing buffer and rLp-PLA activity ranging from 113 to 315 nmol/min/mL. Each run of measurements was performed in duplicate using reagents from three kit lots, with the performance of two runs per day for a 20 day period, yielding 80 results for each kit lot. To determine inter-laboratory (site-to-site) reproducibility, different operators at three independent laboratories assayed Lp-PLA₂ activity in the same set of four K₂-EDTA and four serum specimens with Lp-PLA₂ activity ranging from 22 to 380 nmol/min/mL. Assays were performed at each laboratory daily for five days over a two week period using the same kit lot.

2.11. Method comparisons

To assess the performance of the Lp-PLA₂ activity assay on different chemistry analyzers, 40 specimens with Lp-PLA₂ activity values ranging from 5 to 365 nmol/min/mL were tested on various analyzers optimized with instrument specific settings. Data for each analyzer were compared to those yielded by the Beckman Coulter AU400 analyzer by Deming regression analysis [16].

2.12. Statistical analysis

To determine the limit of quantitation (LOQ), precision profiles using CLSI EP17-A2 guidelines [13] and a target coefficient of variation (CV) of 20% were used. To determine assay linearity, Lp-PLA₂ activity results were compared to expected values using linear regression, and the degree of non-linearity was determined for each data set by second and third order polynomial regression plots using CLSI EP06-A guidelines [15]. A t-test was performed to determine the statistical significance of the non-linear coefficients for selection of best polynomial fit based on the lowest standard error of regression. To calculate deviation from linearity for each concentration, the best fit linear y-value was subtracted from the best fit polynomial y-value at that level with conversion of a difference to a percent value. Matrix and method comparison studies were analyzed by Deming regression [16].

To estimate the variance components for each parameter in the precision analyses, the restricted maximum likelihood (REML) method and a random effects ANOVA model were used, with the latter performed separately for each kit lot. To evaluate site-to-site precision, analyses were performed using JMP software (version 9.0.2) and nested linear components of variance model including two factors, "site" and "day," with "day" nested within "site" following CLSI EP05-A3 guidelines [17]. For the cut-point determination, the JUPITER investigators assessed the cardiovascular risk by Cox proportional hazard regression to calculate hazard ratios across quartiles for Lp-PLA₂ Activity by a model minimally adjusted for LDL-cholesterol and a multivariate model adjusted for age, sex, smoking status, family history of premature atherosclerosis, body mass index, systolic blood pressure, HDL-cholesterol and hsCRP.

3. Results

3.1. Analytical performance

Determination of the expected value ranges for Lp-PLA₂ activity for the general population was performed using archival K₂EDTA plasma specimens obtained from 300 random subjects with age ranging from 35 to 75 years (median = 55.7 years). The population evaluated included a gender distribution of 51% men and 49% women and a racial distribution of 38% Caucasian, 32% African-American, 21% Hispanic and 9% Asian subjects. The central 95th percentile distribution of Lp-PLA₂ activity was 84 to 303 nmol/min/mL for all subjects, and 92 to 329 nmol/min/mL or 74 to 300 nmol/min/mL for men or women, respectively, as shown in Table 1.

Analysis of plasma specimens from 5446 subjects associated with primary prevention of CHD in the placebo arm of the JUPITER Study [14] demonstrated that Lp-PLA₂ activity in the highest quartile was associated with a hazard ratio (HR) for CHD events of 2.83 (CI. = 1.63–4.94) or 2.15 (CI. 1.13–4.08) in minimally-adjusted or fully-adjusted models, respectively. These data enabled the derivation of a cut-point of 225 nmol/min/mL to differentiate those at either increased, i.e., high Lp-PLA₂ ≥225 nmol/min/mL, or decreased CHD event risk, i.e., low Lp-PLA₂ <225 nmol/min/mL risk for CHD events. Use of the cut-point identified 82.0% or 18.0% of the 300 subjects with low or high activity, respectively; similarly, the cut-point identified 79% and 85% of men and women, respectively, with low activity, and 21% and 15% of men and women, respectively, with high activity. No clinical information, i.e., health or disease status, was available for the analysis.

Further analyses yielded an LOQ of 0.4 nmol/min/mL, an LOD of 0.74 nmol/min/mL, and an LOQ of 1.3 nmol/min/mL. Use of weighted linear regression to analyze assay linearity yielded a range of 6 to 382 nmol/min/mL, as shown in Table S1, with deviation from linearity less than or equal to 10%. Assessment of intra-laboratory variability using three kit lots during a 20 day period in the Lp-PLA₂ activity range of 113 to 315 nmol/min/mL yielded the following within-laboratory composite CVs: within-run, ≤1.5%; between-run, ≤1.8%; day-to-day, ≤1.5%;
3.2. Sample matrix

To assess potential variability associated with blood specimen types or plasma collection protocols, Lp-PLA2 activity was measured using matched serum and plasma specimens, as well as matched plasma specimens in different blood collection tubes. As shown in Fig. 2, matched serum and K$_2$EDTA plasma data from 131 subjects demonstrated a slope of 1.00 (95% C.I.: 0.974 to −1.029) in the range of 56 to 357 nmol/min/mL, with an intercept of 0.05 (95% C.I.: -1.28 to 1.39) and an R coefficient of 0.988 by Deming regression analysis [16]. These data demonstrated comparable Lp-PLA2 activity results for matched serum or plasma specimens.

To assess the effect of using different blood collection tubes, Lp-PLA2 activity was measured in specimens obtained from 21 subjects distributed among tubes containing K$_2$EDTA, K$_2$EDTA gel, K$_3$EDTA, and lithium heparin for plasma, and also serum tubes with and without separator gel. Comparison of the matched K$_2$EDTA gel, K$_2$EDTA, and lithium heparin results with those for K$_2$EDTA or serum showed slopes ranging from 1.00 to 1.03 with intercepts ranging from −1.03 to 0.93 and R coefficients ranging from 0.997 to 0.999, thus demonstrating the independence of the Lp-PLA2 activity result from blood collection tube type, as shown in Table S2.

3.3. Sample storage

Since some enzymatic proteins demonstrate lability during storage, the potential variability in Lp-PLA2 activity results due to specimen storage conditions over time was evaluated. Assessment of K$_2$EDTA plasma specimens at 4 °C for up to three months and at 26 °C for up to one month demonstrated average recovery values of greater than or equal to 95% for Lp-PLA2 activity compared to those at baseline, as seen in Table 2. Lp-PLA2 activity ranged from 62 to 239 nmol/min/mL. Evaluation of frozen storage conditions for K$_2$EDTA plasma obtained from 10 subjects and stored at −20 °C or −70 °C for durations of up to 26 months showed differences of less than 10% upon comparison of the average recovery at each time point compared to the baseline value, as shown in Table 2. No statistically significant differences were observed for values between serum and K$_2$EDTA plasma after frozen storage (data not shown). Similarly, serum and K$_2$EDTA plasma specimens (n = 20) subjected to seven freeze–thaw cycles demonstrated average recovery of 95 to 100% at −20 °C and 96 to 97% at ≤−70 °C compared to baseline Lp-PLA2 activity values.

3.4. Substance interference

Since patients with CHD commonly have associated conditions and use medications as part of routine management, the potential variability

![Figure 1](Image) Comparison of Lp-PLA2 activity measurements without and with rLp-PLA2 calibration. Lp-PLA2 activity was determined for 139 serum specimens using two different substrate lots in the absence of rLp-PLA2 calibration. Results were converted to activity units with a factor derived from a non-enzymatic measurement using 4-nitrophenol, as previously described [6,20,21]. Results showed an 18% difference in measurements; no statistically significant difference was observed between the lots using rLp-PLA2 calibration, as shown in Fig. 1.

![Figure 2](Image) Comparison of K$_2$EDTA and serum Lp-PLA2 activity measurements. Lp-PLA2 activity measurements (nmol/min/mL) were determined for 131 matched K$_2$EDTA plasma and serum samples from individual donors and plotted as shown. The data demonstrated a slope of 1.00, an intercept of 0.05 nmol/min/mL, and an R value of 0.988 by Deming regression analysis [16].

Table 2. Lp-PLA2 activity stability by storage conditions.

<table>
<thead>
<tr>
<th>Time</th>
<th>2–8 °C (n = 26)</th>
<th>26 °C (n = 26)</th>
<th>−20 °C (n = 10)</th>
<th>−70 °C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>100%</td>
<td>103%</td>
<td>102%</td>
<td>100%</td>
</tr>
<tr>
<td>Day 7</td>
<td>95%</td>
<td>102%</td>
<td>97%</td>
<td>98%</td>
</tr>
<tr>
<td>Month 1</td>
<td>95%</td>
<td>102%</td>
<td>106%</td>
<td>106%</td>
</tr>
<tr>
<td>Month 2</td>
<td>96%</td>
<td>78%</td>
<td>91%</td>
<td>90%</td>
</tr>
<tr>
<td>Month 3</td>
<td>97%</td>
<td>70%</td>
<td>101%</td>
<td>102%</td>
</tr>
<tr>
<td>Month 13</td>
<td>NA</td>
<td>NA</td>
<td>98%</td>
<td>94%</td>
</tr>
<tr>
<td>Month 26</td>
<td>NA</td>
<td>NA</td>
<td>95%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Average recovery values are shown for Lp-PLA2 activity measurements for the indicated temperatures and time intervals expressed as a percentage of baseline values. (NA, not applicable).
of Lp-PLA₂ activity due to both endogenous and exogenous substances was evaluated. As shown in Table S3, none of the blood components or medications assessed, including those related to CHD, demonstrated interference as shown by analyses of average recovery for spiked and corresponding control samples.

3.5. Inter-laboratory performance

To assess the inter-laboratory variability associated with the Lp-PLA₂ activity assay, site-to-site reproducibility for three different laboratories was evaluated. Site-to-site reproducibility in the Lp-PLA₂ activity range of 22 to 380 nmol/min/mL during a 5 day period yielded individual within-lab precision CVs of 1.4 to 4.2%, as shown in Table 3. Analysis of site-to-site precision demonstrated CVs ≤ 3.4% for all samples and total precision CVs ranging from 2.7 to 4.6%.

Since the Lp-PLA₂ activity assay kit may be used in a variety of laboratory settings, its reproducibility was assessed by testing 40 samples on different automated chemistry analyzers and comparing the results to those observed for the Beckman Coulter AU400 analyzer. As seen in Table 4, method comparison analyses of the analytical performance of the Lp-PLA₂ activity assay using 11 additional chemistry analyzers demonstrated slopes between 0.96 and 1.06 with correlation coefficients (R²) greater than or equal to 0.997. Analysis of once opened and stored, capped reagents at 4 °C from three kit lots at 16 weeks demonstrated average percent recovery ranging from 90 to 110% compared to baseline values. Similarly, use of reagents that were stored uncapped on-board automated clinical chemistry analyzers for 6 weeks demonstrated average recovery ranging from 90 to 110% compared to baseline values.

4. Discussion

Clinical interpretation of Lp-PLA₂ enzyme activity measurements in the management of patients at risk for CHD may be optimized by assay standardization to enhance inter-laboratory agreement and correlation of individual patient results to those observed in published clinical performance data. The clinical use of enzyme activity measurements requires optimization by assay standardization due to myriad contributors to results variability [7], including the following: individual or population heterogeneity phenotypes; specimen collection, handling and storage protocols; assay interference by endogenous or exogenous substances; use of different analytical platforms; differences in raw materials; and differences in laboratory protocols. To enable standardization of measurements of Lp-PLA₂ enzyme activity for patient management, the PLAC Test for Lp-PLA₂ Activity kit includes ready-to-use reagents and a recombinant Lp-PLA₂ (rLp-PLA₂) enzyme for use as calibrators and controls. In the present study, we demonstrate that using rLp-PLA₂ calibrators traceable and consistent to reference material quantitate reproducible Lp-PLA₂ activity assay results, enabling use of patient specimens from different medical practice environments and performance on different automated chemistry analyzers in diverse clinical laboratory settings.

The Lp-PLA₂ enzyme activity assay demonstrated analytical performance sufficient for correlating patient results to data in published clinical studies of Lp-PLA₂ activity in CVD [14]. The analyses yielded an LOQ of 50 nmol/min/mL for analytical sensitivity, which is sufficient to support clinical measurements observed for the CHD population, and an assay linearity range of 5 to 382 nmol/min/mL, which is similar to that reported in an independent analysis [18]. The central 95th percentile of expected Lp-PLA₂ activity values ranged from 84 to 303 nmol/min/mL, with a higher median value of 176 nmol/min/mL observed for men compared to the 154 nmol/min/mL observed for women. Use of an enzyme activity cut-point of 225 nmol/min/mL to determine low (below cut-point) or high (at or above cut-point) activity levels yielded a distribution of 82% and 18%, respectively, for the population studied, while distributions of low and high levels for men or women were 79% or 85% and 21% or 15%, respectively. Comparisons of Lp-PLA₂ activity measurements using two substrate lots, without or with rLp-PLA₂ calibrators, demonstrated that rLp-PLA₂ calibration increased assay reproducibility (Fig. 1). Interference testing demonstrated that neither endogenous circulating substances nor medications typically used by patients at risk for CHD altered Lp-PLA₂ activity results, as shown in Table S3. While differences in blood matrices may influence activity results of enzymes, neither use of serum or plasma, nor blood collection in different tube types yielded statistically significant differences in Lp-PLA₂ activity results. Variations in sample handling with respect to storage in unfrozen or frozen conditions for clinically relevant time periods did not yield statistically significant differences in Lp-PLA₂ activity measurements. Moreover, Lp-PLA₂ enzyme activity measurements remained stable after multiple freeze–thaw cycles. Therefore, potential variations due to pre-analytical specimen handling do not affect calibrated Lp-PLA₂ enzyme activity measurements, in contrast to a prior report for determinations of Lp-PLA₂ concentration by ELISA [19].

Analyses of assay performance on different automated chemistry analyzers with ready-to-use reagents in different laboratories also demonstrated that calibrated Lp-PLA₂ enzyme activity measurements achieved requirements necessary for inter-laboratory agreement and standardized interpretation of activity results stipulated by the International Federation of Clinical Chemistry and Laboratory Medicine [7]. Comparison studies of Lp-PLA₂ activity results performed on multiple automated chemistry analyzers demonstrated Deming regression line slopes of 0.96–1.06 and correlation coefficients greater than or equal to 0.997, thus supporting the independence of the measurements from variation in accuracy due to analysis on various analyzers commonly used in different laboratories. The demonstration of CVs less than or equal to 3.4% for all samples tested on the same analyzer in three different laboratories by different operators also showed the site-to-site reproducibility of calibrated Lp-PLA₂ activity results. The contribution of ready-to-use

<p>| Table 3 | Analysis of intra-and inter-laboratory precision for K₂EDTA plasma and serum specimens. |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (nmol/min/mL)</th>
<th>Within-lab CV</th>
<th>Site-to-site CV</th>
<th>Total CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma 1</td>
<td>21.9</td>
<td>2.6%</td>
<td>2.7%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Plasma 2</td>
<td>108.3</td>
<td>2.1%</td>
<td>2.8%</td>
<td>3.5%</td>
</tr>
<tr>
<td>Plasma 3</td>
<td>233.6</td>
<td>1.8%</td>
<td>1.9%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Plasma 4</td>
<td>379.5</td>
<td>1.4%</td>
<td>2.3%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Serum 1</td>
<td>213.6</td>
<td>1.7%</td>
<td>1.9%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Serum 2</td>
<td>102.3</td>
<td>2.1%</td>
<td>3.4%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Serum 3</td>
<td>232.3</td>
<td>2.1%</td>
<td>3.1%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Serum 4</td>
<td>355.9</td>
<td>2.3%</td>
<td>2.2%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

Shown are the mean Lp-PLA₂ activity results, the within-lab coefficient of variation (CV), the site-to-site CVs using the Beckman Coulter AU400 analyzer at three independent sites, and the total CV for each of four K₂EDTA plasma and four serum specimens.

| Table 4 | Method comparison of automated clinical chemistry analyzers. |
|---------|-----------------|---------------|----------|
| Clinical chemistry analyzer | Slope | Intercept | R² value |
| Abbott ARCHITECT c8000/ci8200 | 1.06 | −3.47 | 0.999 |
| Beckman Coulter AU480 | 1.00 | −3.35 | 0.999 |
| Beckman Coulter AU640 | 0.96 | 2.03 | 0.999 |
| Beckman Coulter AU5800 | 0.99 | −0.90 | 0.999 |
| Beckman Coulter DxC600 | 1.01 | −2.38 | 0.999 |
| Beckman Coulter DxC800 | 1.03 | −4.73 | 0.999 |
| Roche cobas 6000 (c501) | 1.03 | −4.48 | 0.999 |
| Roche cobas 8000 (c502) | 1.05 | −3.31 | 0.997 |
| Roche Modular-P | 1.05 | −5.62 | 0.999 |
| Siemens Advia 1800 | 0.99 | 1.74 | 0.998 |
| Siemens Advia 2400 | 0.96 | 0.58 | 0.999 |

Shown are the slopes, intercepts (nmol/min/mL), and R² coefficient values for Lp-PLA₂ activity results obtained using the eleven indicated clinical chemistry analyzers with data for each compared to those obtained using the Beckman Coulter AU400 analyzer.
The clinical value of a standardized Lp-PLA2 enzyme activity assay is demonstrated by a recombinant Lp-PLA2 protein with ready-to-use reagents to achieve high intra-laboratory and inter-laboratory precision, and also the requirements stipulated for enzyme activity assay standardization [7]. The data also demonstrate that a calibrated Lp-PLA2 activity measurement is independent of heterogeneity in the therapeutic management of patients at risk for CVD and in pre-analytical processes such as blood collection and handling common to diverse practice settings. The clinical value of a standardized Lp-PLA2 enzyme activity assay is that it may 1) enhance physician interpretation of activity measurements by enabling correlation of an individual patient’s result to extensive, published clinical validation data for CVD population outcomes, and 2) enables harmonization of results obtained on patients from different laboratories with varying operating protocols.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2015.11.018.

References